Isoflavones from Abrus mollis

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Abstract: **Object** To study the chemical constituents from the whole plant of Abrus mollis **Methods** Chemical constituents were isolated by column chromatographic methods And their structures were elucidated by spectroscopic methods **Results** One isoflavone glycoside and three isoflavones were purified and determined as 8-methylretusin-7-O-β-D-glucopyranoside (Ⅳ), retusin 8-methyl ether (Ⅱ), 4', 7, 8-trimethoxyisoflavone (Ⅲ), and afrormosin (Ⅵ). **Conclusion** All of these four compounds are first isolated from the plant of A. rnollis

Key words: Abrus mollis Hance; isoflavone; isoflavone glycoside

毛相思子中的异黄酮类成分

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摘 要: **目的** 研究毛相思子全草的化学成分。**方法** 用柱色谱法分离得到化学成分, 用光谱法测定其结构。**结果** 纯化出一个异黄酮苷和 3 个异黄酮成分, 分别是 8-methylrelusin-7-O-β-D-glucopyranoside (Ⅳ)、retusin 8-methylether (Ⅱ)、4', 7, 8-trimetlloxyisoflavone (Ⅲ) 和 afrormosin (Ⅵ)。**结论** 这 4 个成分均为首次从该植物中分得。

关键词: 毛相思子; 异黄酮; 异黄酮苷
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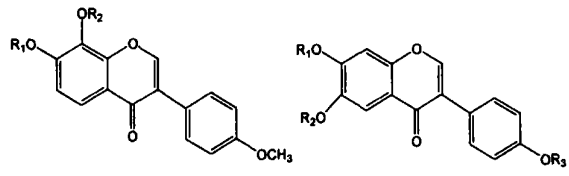
The whole plant of *Abrus mollis* Hance was used to treat hepatitis and pediatric dyspepsia, as well as externally used for burn injuries in skin[1]. In the region of Southwestern China, *A. mollis* is regarded as an alternative of *A. precatorius*, a recognized medicinal herb in traditional Chinese medicine. The preparation containing *A. precatorius* has been embodied in 1977 version of *China Pharmacopoeia*. Although *A. precatorius* has been extensively explored and many alkaloids, i. e. terpenoids, steroids, flavanoids, amino acids, and saccharides isolated from this plant[2,3], phytochemical studies of *A. mollis* have not been reported in literatures prior to the identification of eight compounds, they are triterpenoids, fatty acid, and fatty acid methyl ester from this plant[4]. Structure elucidation of one isoflavone glycoside, namely 8-*O*-methylretusin-7-*O*-β-*D*-glucopyranoside (Ⅳ), as well as three isoflavones, retusin 8-methyl ether (Ⅱ), 4′, 7, 8-trimethoxyisoflavone (Ⅲ), and afrormosin (Ⅵ) were presented here. Structures Ⅰ—Ⅸ are seen in Fig. 1.



Ⅰ $R_1=H, R_2=H$
Ⅱ $R_1=H, R_2=Me$
Ⅲ $R_1=Me, R_2=Me$
Ⅳ $R_1=\beta$-D-Glu, $R_2=Me$
Ⅴ $R_1=\beta$-D-Glu, $R_2=H$
Ⅵ $R_1=Me, R_2=H, R_3=Me$
Ⅶ $R_1=H, R_2=H, R_3=Me$
Ⅷ $R_1=Me, R_2=H, R_3=H$
Ⅸ $R_1=H, R_2=Me, R_3=H$

Fig. 1 Structures of compounds Ⅰ—Ⅸ

1 Apparatus and materials

Melting points of the compounds were determined with a Germany—68992 apparatus. IR spectra were recorded with a IR—47 spectrometer. NMR spectra were measured with a Varian INOVA—500 spectrometer, using TMS as internal

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standard. A VGZAB－HS and a QB－200 mass spectrometer were used to record the MS data. Silica gel (100－200 meshes) and silica gel H (Qingdao Marine Chemical Group Co.) were used for column chromatography and TLC, respectively.

A. mollis was collected at Yunlin of Guangxi Zhuang Autonomous Region in October 2001, and identified by QIN Dehai of Guangxi Institute of Traditional Medical and Pharmaceutical Sciences. The authentical sample of the plant was deposited at the Herbarium of the same institute.

2 Extraction and isolation

The whole herbs of *A. mollis* (25.32 kg) were extracted with 15 times of volume of 95% ethanol continuously. The ethanolic extracts were concentrated *in vacuo* to yield 1 778 g of brown viscous extracts, which were further partitionated with petroleum ether (60－90 ℃), chloroform, and ethyl acetate successively. The chloroform fraction was subjected to column chromatography on silica gel (100－200 meshes) with a $CHCl_3$-MeOH gradient system repeatedly to afford compounds Ⅱ－Ⅳ and Ⅵ.

3 Identification

Compound Ⅳ: $C_{23}H_{24}O_{10}$, white prisms; FAB－MS m/z: 461 ($[M+H]^+$); ^{13}C-NMR are seen in Tables 1 and 2; and the unsaturated number was thus calculated as 12; ^{1}H-NMR, δ 7.95, as well as other aromatic signals sugested a multi-oxygenated isoflavone structure for Ⅳ. A pair of doublets at δ 7.92 (1H, d, J=9.0 Hz) and δ 7.37 (1H, d, J=9.0 Hz) showed the presence of the ortho aromatic protons arising from dimethoxy substitution on ring A, and the δ 7.92 doublet was assigned to H-5 by comparing the spectral data of Ⅳ with those of compound Ⅰ－Ⅲ[5,6] and Ⅴ[7]. In addition, a pair of doublets at δ 7.00 (1H, dd, J=6.5, 2 Hz) and δ 7.52 (1H, d, J=6.5, 2 Hz) with four protons indicated the 4′-oxygenated B ring. The signals for five protons between 3.4－3.9, together with a doublet at δ 5.18 (J=6.0 Hz) suggested the presence of aβ-*D*-glucosyl moiety. The presence of HMBC correlation between the proton signal δ_H 5.18 (H-1′) and C-7 indicated that the glucosyl was attached to C-7 of the aglycone. Two methoxy groups at δ 3.78 and 3.93 were assigned to 8 and 4′ positions accordingly. On acid hydrolysis, Ⅳ gave *D*-glucose. Compound Ⅳ was thus elucidated as 8-*O*-methylretusin-7-*O*-β-*D*-glucopyranoside[12]. As the white prisms (anhydrous ethanol), mp 216－218 ℃. IR ν_{max}^{KBr} cm^{-1}: 3 470, 1 637, 1 604, 1 570, 1 449, 1 397, 1 284, 1 247, 1 058, 1 022. FAB-MS m/z: 461 ($[M+H]^+$, 9%), 307 (10%), 299 (24%), 154 (100%), 136 (83%), 107 (31%). ^{1}H-NMR and ^{13}C-NMR are seen in Tables 1 and 2.

Compound Ⅱ: The spectral data of compound Ⅱ was almost identical to those of the aglycone part of compound Ⅳ. The spectral data of Ⅱ was agreement with the retusin-8-methyl ether. Subsequently, Ⅱ was identified as retusin-8-methyl ether. As the yellow needle (petroleum ether-chloroform), mp 231.5－232.5 ℃. IR ν_{max}^{KBr} cm^{-1}: 3 510, 3 240, 1 634, 1 604, 1 570, 1 507, 1 446, 1 311, 1 289, 1 246, 1 034, 989. FAB-MS m/z: 299 ($[M+H]^+$), 154, 136, 107, 89, 77. ^{1}H-NMR and ^{13}C-NMR are seen in Tables 1 and 2.

Table 1 ^{1}H-NMR spectral data of isoflavones Ⅱ－Ⅳ and Ⅵ (DMSO-d_6)

Proton	Ⅰ	Ⅱ	Ⅳ	Ⅵ
H-2	7.95 s	8.43 s	8.24 s	7.93 s
H-5	7.71 d (8.5)	7.60 d (8.7)	7.92 d (9.3)	7.66 s
H-6	7.02 d (8.5)	7.22 d (9.0)	7.38 d (9.3)	
H-8				6.98 s
H-3′,5′	6.98 d (9.0)	6.99 d (8.7)	6.99 d (8.7)	6.98 d (8.7)
H-2′,6′	7.49 d (8.5)	7.52 d (8.4)	7.49 d (8.7)	7.51 d (8.7)
OMe	3.86 (8-OMe)	3.93	4.04 (4′-OMe)	4.02 (6-OMe)
	3.77 (4′-OMe)	3.78	3.84 (8-OMe)	3.84 (4′-OMe)
		3.36		
H-1″			5.13 d (6.0)	
H-2″,6″			3.46～3.92 m	
OH				6.27 brs

* Coupling constant J (in brackets) in Hz

Compound Ⅲ: Pale yellow prisms (petrollum ether-chloroform), mp 194－196 ℃. ^{1}H-NMR are seen in Table 1. So Ⅲ was identified as 4′, 7, 8-trimethoxyisoflavone by comparison with literatures[5], and with the spectral data of Ⅰ and Ⅱ.

Table 2 DEPT, ^{13}C-NMR, ^{1}H-NMR, and HMBC spectral results of Ⅱ and Ⅳ (DMSO-d_6)

Position	DEPT	Ⅱ			Ⅳ		
		^{13}C-NMR	^{1}H-NMR	HMBC	^{13}C-NMR	^{1}H-NMR	HMBC
2	C	153.14	7.95 s	C-4, C-9, C-3	153.70	8.24 s	C-4, C-9, C-3
3	C	123.00			123.14		
4	C	174.82			174.88		
5	CH	120.82	7.71 d	C-4, C-7, C-9	120.36	7.92 d	C-4, C-7, C-9
6	CH	115.21	7.02 d	C-8, C-10, C-7	114.30	7.38 d	C-8, C-10, C-7
7	C	154.70			154.09		
8	C	134.70			136.90		
9	C	150.72			150.06		
10	C	117.51			119.36		
1′	C	124.17			123.98		
2′,6′	CH	130.15	7.49 d	C-4′, C-3′/5′, C-3	130.75	7.49 brd	C-1′
3′,5′	CH	113.67	6.98 d	C-4′, C-2′/6′, C-1′	113.73	6.99 brd	C-2′/6′, C-4′
4′	C	159.03			159.10		
8-OMe	CH_3	60.85	3.86 s	C-8	61.31	3.84 s	C-8
4′-OMe	CH_3	55.19	3.77 s	C-4′	55.21	4.04 s	C-4′
1″	CH				100.47	5.13 d	C-7
2″,5″	CH				77.24,76.57	3.19～3.70 m	
3″	CH				73.20		
4″	CH				69.49		
6″	CH_2				60.50		

* Coupling constant J (in brackets) in Hz

Compound Ⅵ: It was elucidated as a dimethoxy isoflavone due to its spectral similarity to those of Ⅶ — Ⅸ[8~10]. Three singlets at δ 7.93, 7.66, and 6.98 indicated that the A ring is substituted by oxygenated groups at C-6 and 7, and were thus assigned to characteristic H-2 signal as well as H-5 at lower field and H-8 signals. The δ 7.66 signal was assigned to H-5 since it was deshielded by carbonyl group at chromone ring, and the δ 6.98 signal was located at H-8. One methoxyl group at 4.02 was assigned to C-6 because it showed strong NOE with H-5. In addition, a NOE was observed between 4′-methoxyl group protons and 3′ and 5′ protons, conforming that another methoxyl group was linked to C-4′. In a word, the proton signals of ^{1}H-NMR spectra of Ⅵ were in accordance with those of afrormosin[11]. As the yellow to pale brown needles (anhydrous ethanol), mp 231—232 ℃. FAB-MS m/z: 299 ([M+H]$^+$, 22%), 279 (20%), 154 (100%), 136 (100%), 107 (35%), 89 (37%), 77(37%).

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